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TRANSACTIONS
of
THE NEW YORK ACADEMY OF SCIENCES

Ser. II, Vol. 4 DECEMBER, 1941 No. 2

SECTION OF BIOLOGY*

NOVEMBER 10, 1941

SYMPOSIUM ON ALLERGY

BRET RATNER AND HAROLD A. ABRAMSON

I

DOCTOR BRET RATNER, New York University College of Medicine, New York, N. Y.: *The Nature of Allergy.* (This lecture was illustrated by motion pictures.)

At the dawn of the twentieth century, horse serum in the form of diphtheria antitoxin was just beginning to be used. Practically all children who received such antitoxin developed, after a week or more, a condition to which Von Pirquet gave the name "serum krankheit." The symptoms presented were urticaria, angioneurotic edema, temperature and certain blood changes. The condition lasted for a few days and, in most instances, passed off without untoward results. However, Pirquet noted that, when repeated injections were administered to these same children at subsequent times, some developed asthmatic seizures along

*The Section of Geology and Mineralogy met November third. No abstract was received from Doctor Paul F. Kerr, who gave an address entitled, "A Brazilian Reconnaissance."

TRANSACTIONS of The New York Academy of Sciences, Series II, Volume 4, No. 2, December, 1941.
This publication is distributed to Members and is published monthly from November to June, inclusive, at 109 West Chestnut Street, Lancaster, Pa., by The New York Academy of Sciences, Seventy-ninth Street and Central Park West, New York City.
Associate Editor: Eunice Thomas Miner, Executive Secretary.
Entered as second-class matter December 2, 1938, at the post office at Lancaster, Pa., under the act of August 24, 1912.

with the symptoms mentioned above, which came on precipitously within a short interval after the injection. He realized that he was dealing with a new entity. Somehow these children were changed by their first injection and they reacted in an altered manner to subsequent injections.

Because the term which Pirquet coined "allergy" has been popularized, I have cited his work first. But, a few years prior (1902), a French physiologist, Charles Richet, made the following remarkable observation. He was experimenting upon dogs with an extract of certain sea fauna. He injected one particular dog, whose name was Neptune. This dog was unaffected by the first injection. Professor Richet then went off on a holiday. Upon his return, some 22 days later, he again injected Neptune with the same extract. To his amazement the dog rapidly went into shock, had a hemorrhage from the bowels, vomited copiously and died within a few minutes. He had injected many animals prior to this, but never before had such a thing happened. He retraced his steps and came to the realization that he had fallen upon the discovery of a heretofore unsuspected biological phenomenon, namely, that an animal that is injected with an otherwise harmless substance is changed somehow in his reaction to a subsequent injection of this same substance. Richet called this phenomenon anaphylaxis.

That Pirquet did not credit Richet with his discovery and adopt the term anaphylaxis for the phenomenon in the human being was a source of great unhappiness to the French physiologist. In the voluminous literature that has stemmed from these original discoveries, his failure to do so has given rise to a great controversy which still is unsettled. Are anaphylaxis in the animal and allergy in man identical phenomena? The controversy still rages and, while I, personally, feel it has impeded our progress in this field, nevertheless we have moved on.

It is probably fitting, before showing the film, to recall in passing that Richet, in his altogether delightful volume "French Savants," remarked that one can work a lifetime on a problem and not contribute anything of significance, and yet make a discovery

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of great importance after working on another problem for but a short period. Thus was anaphylaxis discovered, a phenomenon in biology which bids fair to take its place amongst the great discoveries, and for which Richet deservedly got the Nobel Prize.

With these all but too few remarks, I should like now to present my moving picture, which may help to correlate the observations thus far made in man and animal.

The following is a synopsis of the cinema as it unfolds on the screen:

The entrance of harmless foreign proteins (antigens) into the body of man or animal may give rise to a state of hypersensitiveness. The initial entrance of a foreign protein produces no ill effects. Following an incubation period of at least 10 days, a second entrance or reinjection of the same protein may no longer be innocuous, but may produce severe reactions. Because it is capable of inducing a state of hypersensitiveness, the *primary* contact is known as the *sensitizing dose*. The *secondary* or subsequent contacts are known as *shock doses*. The terms most commonly used to describe the phenomenon of protein hypersensitiveness are *anaphylaxis* and *allergy*. *Anaphylaxis* means "without protection" and usually refers to the shock reactions in experimental animals, but may also be used to denote severe reactions and death in man. *Allergy* means altered "reactivity" and, in man, encompasses a large variety of hypersensitive states and syndromes clinically manifested in the form of *asthma, hay fever, eczema, urticaria, angioneurotic edema, migraine, gastrointestinal disturbances*, and possibly other disease entities. Allergy is the more inclusive term, for it not only includes anaphylaxis and the allergic diseases, but also a variety of conditions found in tuberculosis, scarlet fever, rheumatism, and other infectious diseases. Knowledge of allergy depends, probably more than that of any other malady, on animal experimentation.

Anaphylaxis in Experimental Animals

I. The Guinea Pig. Normal guinea pigs are given a sensitizing injection of a foreign protein by the intraperitoneal and

subcutaneous routes. They remain normal. Two weeks later, sensitized animals are given a second or shock injection of the same protein by the intravenous route. Mild reaction. Lethal anaphylaxis. The pathology of anaphylaxis in the guinea pig is essentially a tetanic spasm of the terminal bronchioles which results in emphysema. Comparison with the normal lung. A diagram of the bronchial tree (shown on the screen) illustrates the pathology of anaphylaxis. The spasm of the terminal bronchiole is the direct cause of the symptoms. The explanation of this phenomenon lies largely in the *cellular theory*, which states that, after an antigen has entered the body and a suitable incubation period has elapsed, specific anti-bodies are formed and become fixed to tissue cells. Like Ehrlich's graphic hypothesis of immunity, the following symbolism enables us to grasp somewhat the concept underlying hypersensitiveness. It is admittedly hypothetical and, from the physicochemical viewpoint, inadequate. This represents a smooth muscle cell. When antigen enters the blood stream, it remains there for a short time and then disappears. After a period of 10 days to 2 weeks specific antibodies are presumably attached to the smooth muscle cells. When the specific antigen again enters the blood stream a union occurs between it and the fixed antibodies of the unprotected cell. This union of fixed antibodies and antigen initiates the sequence of events which results in the characteristic allergic response. The validity of this theory is greatly fortified by the fact that the uterine muscle of a sensitized animal *when isolated from the organism* still shows a definite allergic reaction to the specific antigen. The following kymogram shows the effect of the antigen on the normal and regular contractions of the uterine strip. *The animal was sensitized with egg white* two weeks previously. 1. Minute markings on kymograph tracing. 2. Normal uterine irritability. 3. Normal uterine waves. 4. Milk added to bath—no reaction. 5. Egg white added to bath. 6. Allergic uterine contraction. 7. Height of contraction. 8. Tetanic spasm. 9. Relaxation of muscle. 10. Fresh Locke's solution added. 11. Egg white added to fresh bath. 11A. No reaction—desensitized muscle. 12.

Histamine added. 13. Non-specific uterine contraction. 14. Relaxation. The same action that causes the tetanic contraction of the uterine muscle, lasting for several minutes, is sufficient to cause the death of an animal when the *bronchioles* are constricted. The physiological pathology of anaphylaxis depends entirely upon the contractility of smooth muscle, and the specific symptoms of anaphylaxis will vary from species to species. The predominating symptoms in each species are accounted for by the location of the largest amount of smooth muscle present in the different vital organs; e.g., the *bronchioles* in the guinea pig, the *pulmonary artery* in the rabbit, the *hepatic venules* in the dog.

II. The Rabbit. A normal rabbit is given an intravenous injection of a foreign protein and shows no reaction. A sensitized rabbit is given a shock injection. The symptoms are mild and are due to a constriction of the pulmonary artery. It is difficult to induce severe anaphylaxis in the rabbit. The rabbit develops a higher precipitin titer than any other animal, which can easily be demonstrated by the precipitin test. A normal guinea pig readily can be passively sensitized with the blood of a sensitized rabbit. If a rabbit is given subcutaneous injections repeatedly at several day intervals he will develop a local inflammatory reaction in the skin and underlying tissue, known as the *Arthus phenomenon.* This phenomenon has been reported in the human being after repeated injections of serum and may sometimes result in severe local necrosis.

III. The Dog. Dogs were given intravenous injections of 10 cc. of a foreign protein (horse serum), followed three days later by a subcutaneous injection of the same protein. Three weeks later, these dogs are given an intravenous shock injection of 20 cc. The animals show symptoms of anaphylaxis ranging from a mild degree to severe shock terminating in death. Note the retching, vomiting, hemorrhage from the bowel, and complete collapse.

The anaphylactic symptoms were due directly to a constriction of the *hepatic venules.* The first animal recovered and the last died in complete collapse with continued hemorrhage from the bowel (death occurred two hours later). The liver, removed from

the animal which died, weighed 485 gms. Contrast this with a liver removed from a dog of the same size dying of other causes: weight, 130 gms. The enlargement is due to a stasis of portal blood. The sequence of events in canine anaphylaxis can be visualized with the aid of the following diagram. In addition to the blood pressure changes, at the height of anaphylaxis, there are chemical changes in the blood indicated by the loss of coagulability. Blood drawn from the sensitized dog before the shock injection coagulates in several *minutes*. Blood taken from the same dog at the height of anaphylactic shock does not coagulate even after several hours.

Immunity Phase of Hypersensitiveness

When an animal has recovered from anaphylactic shock, it will resist a subsequent injection of the specific antigen. This phase is known as *anti-anaphylaxis*. When an animal has received small daily injections of a specific antigen *without an incubation period*, it will also be refractory. Sensitized animals which receive frequently repeated small injections of the specific antigen may become *desensitized*. These immunity phases are temporary and relative, depending largely upon the quantitative relation of antigen to antibody. A graphic representation of what probably occurs during these periods. During antianaphylaxis and desensitization, antibodies remain fixed to the cells. They are also in excess in the circulation. If antigen enters the blood stream during these periods, it is neutralized by the excess antibodies in the *circulation* and is prevented from reaching and combining with the antibodies in the smooth muscle cells. The proof that human allergy is due to an antigen-antibody complex is furnished by the Prausnitz-Küstner reaction. Prausnitz-Küstner reaction: Step 1—Blood is drawn from an *allergic* child. Prausnitz-Küstner reaction: Step 2—The serum separated from this blood is injected *intradermally* into a *normal* adult. Prausnitz-Küstner reaction: Step 3—48 hours later the protein, to which the allergic child was sensitive, is injected intradermally into the passively sensitized area of the recipient. A positive reaction occurs *in this area*, but

not elsewhere. This passive sensitization of a localized area in the skin of a normal individual is due to the presence of specific antibodies in the blood of the allergic individual.

Naturally Acquired Hypersensitivity

The hypersensitivity of man does not arise exclusively from injection. A protein may enter the body through a *natural portal*, and function as though it were injected. The ways in which a human being may become sensitized can be demonstrated in the guinea pig.

Experimental Asthma. A normal guinea pig is placed for several hours in an atmosphere containing dry horse dandruff. There is no reaction. Three weeks later guinea pigs that have inhaled dry horse dandruff dust for several hours are returned to this same dust environment and show shock symptoms. Sensitization and shock manifestations in these animals were induced solely through the medium of inhalation, showing different degrees of hypersensitiveness. The death of the animal is due to the tetanic spasm of the bronchioles, for the *heart* remains beating several minutes after asphyxia. If an animal is sensitized by the inhalation of horse dandruff, it may die from a *primary injection* of horse serum *antitoxin*. Asthmatic human beings sensitive to horse dandruff may, in like manner, die suddenly after a primary injection of horse serum.

Food Allergy. Normal guinea pigs are fed, for the *first* time, a harmless natural product—milk. No reaction ensues. Two weeks later, when animals, sensitized by feeding, are fed milk for the second time, anaphylaxis may result. About 50% of animals can be sensitized through the medium of feeding. The reactions are usually mild, but infrequently may be severe or fatal.

Fatal anaphylaxis in human infants, following the ingestion of milk, has been reported. These reactions occur with *native* food proteins. When such proteins are *denatured* by heat or digestion they may lose their antigenic properties. For example, *whey proteins* of milk are *most often* responsible for milk idiosyncrasy. Intensive heat treatment under controlled conditions, will

denature *whey* proteins so that such milk can be tolerated by a milk sensitive individual. A guinea pig sensitized to *pure lactalbumin* resists a shock injection of denatured milk; but when this is followed by an injection of *undenatured* raw milk the animal dies in anaphylactic shock. No reaction with denatured milk. Death from raw milk (same animal). Malt extracts contain antigenic barley proteins. Carbohydrates derived from malt extract lose their antigenic properties in the process of preparation. An animal sensitized to *malt extract* is given a shock injection of a 10% solution of *dextri-maltose* and shows *no reaction.* When this is followed by an injection of a 10% solution of malt extract anaphylaxis occurs. No reaction with dextri-maltose. Death from malt extract (same animal).

Sensitization in Utero-Congenital Allergy. If pregnant animals are given either injections of horse serum, or feedings of milk, or inhalations of horse dandruff, their offsprings may become sensitized *in utero.* A newborn, sensitized *in utero,* may show an anaphylactic reaction on first contact with the substance to which it was sensitized. This sensitization *in utero* may be *passive* or *active.* Guinea pigs born of normal mothers show no reaction. A guinea pig, "actively sensitized *in utero,*" is shown to react to its initial contact with horse serum when three weeks old. This experimental demonstration of sensitivity, congenitally acquired, lays the basis for an understanding of certain so-called inherited types of hypersensitivity in human infancy. In these instances, the infant is either actively sensitized to a food protein the pregnant woman has ingested; or, the infant is passively sensitized to the antigen to which the allergic mother reacts.

Human Allergy

Approximately 10% of the population suffer from the major allergies—asthma, hay fever, allergic eczema, or allergic urticaria. A much larger proportion of the population probably have minor allergic manifestations, which would also include certain drug idiosyncrasies. The generally accepted viewpoint is that allergy is inherited. As demonstrated, however, specific hypersensitivi-

ties can be naturally acquired by the individual. The diagnosis of allergic diseases depends to a large extent on a careful history, a complete physical examination, and a thorough differential diagnosis to exclude non-allergic conditions which simulate these diseases. Once it is determined that the patient is allergic, the physician must next embark upon the search for the protein or proteins directly responsible for the allergic symptoms. The specific offender can often be discovered by careful observation and by the use of elimination diets. However, the truly great advance in recent clinical knowledge of the specific causes of allergic diseases has been made by the application of the protein skin tests. Two methods are available: (1) the intracutaneous, and (2) the scratch. The latter method is preferred, particularly in children. A positive skin reaction is one which usually occurs within a few minutes and assumes the form of a wheal with erythema. Like all other laboratory methods the skin test has its limitations: (1) All patients do not give positive reactions even if they are sensitive; (2) Reactions may be positive at one time and negative at another; (3) Reactions may vary at different sites; (4) The interpretation of a positive reaction is not always easy; (5) Delayed reactions may sometimes occur. The larger the number of proteins available for application the more exact will the diagnosis become. Demonstrations of the scratch test: dried proteins are dissolved *in situ* with N/10 NaOH, at the site of the scratch. Treatment is based on the specific protein sensitivity.

It is accomplished in one of the following ways:

1. By a complete elimination of the offending protein from the diet or the environment. This is often a more difficult task than is apparent. Frequently foods, in denatured form, may be used to augment the diet and not deprive the individual of certain important food elements.

2. By establishing a tolerance for the offending protein by *oral desensitization*. Small amounts of food are given in gradually increasing quantities until tolerance is established. This may require from 6 to 9 months.

3. By establishing a tolerance for the offending protein by

parenteral desensitization. Injections are given subcutaneously. The dilution of the protein must be high enough not to produce any reaction in the patient. The increases are very slow and gradual. Great care must be exercised in the quantity administered as severe shock reactions—sometimes fatal—are known to have occurred following the injection of a quantity exceeding the tolerance of the patient.

For the desensitization of the hay fever sufferer, in which the injection method is most often used, there are two choices:

1. The "pre-seasonal" method, i.e., the injection of pollen extract at intervals for several months immediately preceding the allergic season.

2. The "perennial" method, i.e., injecting at regular intervals throughout the year.

The perennial method is the one of choice. Once more the great caution that should be exercised when any foreign protein is injected into a human being should be emphasized, for the very same symptoms that were observed to occur with the animal can occur in the human allergic. Besides the typical guinea-pig type of anaphylactic death in man, there has been a report of an individual who died from a tetanus antitoxin injection who showed all the symptoms and pathology noted in canine anaphylaxis. Serum administration of any kind and injections of foreign proteins such as milk, insulin, and others, must all be given with circumspection. The individual should be previously tested to avoid anaphylactic shock. Animal experimentation has thus thrown light on a large group of human ailments in the domain of protein hypersensitiveness and has established the basis for diagnosis and treatment.

DOCTOR HAROLD A. ABRAMSON, Columbia University and The Mount Sinai Hospital, New York, N. Y.: *The Nature of the Ragweed Pollen Allergen.*[1] (This lecture was illustrated by lantern slides.)

II

The most common manifestations of constitutional allergy in the United States are hay fever and its frequent complication, asthma. To the pollens of the ragweeds and of the grasses may be assigned the most important etiological role in so far as the production of sensitization and tissue reaction in susceptible individuals are concerned. The tiny pollen granules are breathed in through the respiratory tract. During their travels in the nasal and bronchiole tubes of sufficiently sensitized persons (or animals), the pollen collide with the moist mucous membranes and release the active agents, the allergens, at the site of contact. These allergenic substances then pass through the mucous membranes into the underlying tissue where a tissue reaction occurs leading to dilatation of the minute blood vessels and transudation of plasma-like material. In addition, the smooth muscle cells of the bronchioles may respond with the common phenomenon of spasm. It is evident that the exact nature of the molecules which produce these symptoms is of the utmost significance if the mechanisms of the processes involved are to be understood.

It is always of interest to describe the first experiments which led to the development of any investigation. The first experiment which was performed in this series of studies on the nature of the ragweed pollen allergen was an attempt to transport it electrophoretically into the skin of a medical student who was sensitive to ragweed. Much to my surprise, using the negative pole, a large hive with pseudopods formed at the point of application of the ragweed solution. No reaction occurred in individuals not sensitive to ragweed pollen. This was noteworthy, not so much because of the fact that an electrical field could drive substances into the living human skin but because materials presumably of

[1] This investigation has been aided by a grant from the Josiah Macy, Jr. Foundation.

high molecular weight and protein in nature readily penetrated the epidermis and reached the blood vessels in the dermis where the allergic reaction occurs. It was even more unexpected that both the positive and the negative poles were able to introduce the biologically active constituents of ragweed pollen extracts. There was no obvious reason why this should occur. In the case of giant ragweed pollen extracts, the positive pole was more efficacious. In the case of dwarf ragweed pollen extracts, the negative pole proved more efficacious. The fact that biologically active materials may be introduced in this way has been used in the therapy of hay fever both coseasonally and preseasonally.

Similar phenomena were found for timothy pollen extracts.

The fact that the allergens of ragweed and timothy pass through as relatively impermeable a membrane as the intact human skin, makes it simpler to visualize why they readily pass through the mucous membranes of the lungs and the nose. Just why they are apparently able to do this will be disclosed shortly.

Cooke, Stull and their co-workers have shown that the nitrogen precipitated by phosphotungstic acid is an important measure of the biological activity of ragweed and other pollen extracts. These workers have also found, by chemical precipitation technics, two fractions with important immunological properties in ragweed pollen solutions. Furthermore, by the use of dialysis methods and ultrafiltration, Spain and Newell as well as others have emphasized that the colloidal fraction of pollen extracts is, in all likelihood, connected with the measure of their therapeutic efficiency. The work of the writer with Sookne and Moyer also support, the view subscribing to the protein nature of the biologically active agent of ragweed pollen extracts. These investigators found that ragweed extract could be inactivated by adsorption with inert surfaces in very much the same way that serum albumin, egg albumin, and other proteins are adsorbed. Thus, quartz particles inactivated dilute ragweed pollen extract. The electrical properties of the adsorbed film obtained from ragweed pollen extracts were investigated by the microscopic method of electrophoresis.

In view of the fact that crude extracts were employed to obtain adsorption films of the active principle, it was necessary to determine that the constituents adsorbed would be taken out by the adsorbing surface independently of the nature of the surface. For this reason, typical hydrophilic and hydrophobic surfaces were compared in the following way. Electric mobility measurements were made on solutions containing increasing quantities of pollen extracts in 0.02 M phosphate buffer. It was found that the electrical mobilities of mineral oil droplets, collodion particles, and quartz particles in the presence of an excess of pollen extract were the same. This meant that essentially the same film was on these very different surfaces. When quartz particles which had been treated with pollen extract were exposed to varying concentrations of acid, reversal of the sign of charge occurred near pH 4.0 which indicated that the adsorbed film was amphoteric and probably protein-like in nature but not necessarily so. Further experiments on the electrical mobility-pH curve indicated the protein-like nature of the surface, but the electrical mobilities in alkaline solutions were very low with a practically flat mobility-pH curve. The titration curve of a sample of dialyzed ragweed extract also acted as an amphoteric solution reacting with both acids and bases approaching maximum acid combining capacity at approximately pH 2.6. On the basis of these adsorption and electrical experiments on the ragweed pollen material, we were quite ready to believe that we were dealing with a film very much like a protein but with an unusually low and flat mobility-pH curve on the alkaline side of the isoelectric point.

Although the experiments cited in the foregoing shed some light on the nature of the material, it was evident that fractionation of the deeply colored extracts could probably best be achieved by electrophoretic separation employing the moving boundary method. These experiments were begun in the summer of 1938 with J. Gagarin and L. Jennings at the Biological Laboratory, Cold Spring Harbor. Using a conventional U-tube, it was observed that the pigment was negatively charged and that the impure fast moving pigment was skin reactive. During that year,

Gagarin with the collaboration of H. Gettner ran a large number of experiments with the same equipment and noted that some of the colorless material left behind by the migrating pigment was also highly skin reactive. On the basis of these fairly crude experiments with the simple U-tube, then, it was found that biological activity was present both in colorless and pigmented fractions. However, we were unable to obtain any quantitative data on the electrical mobility of the colorless fraction, on the number of pigments, on the types of molecules concerned, or on the homogeneity of the fractions. At this point, the collaboration of D. H. Moore was enlisted and the Tiselius apparatus at the Electrophoresis Laboratory of Columbia University was employed. The use of the electrophoresis equipment and the ultracentrifuge by Dr. Moore has made further important progress possible. The optical method first invented by Foucault for correcting telescope errors and adapted by Toeppler, Lamm and Tiselius for observations of refraction gradients has simplified the problem to the point where an ultimate solution seems likely—indeed most probable.

With ordinary Visking tubing and collodion membranes, we were unable to prevent loss of large amounts of highly active allergenic material. With dried collodion membranes, the diffusion of the allergen could be retarded, but these membranes also prevented the diffusion of salt to a great extent. After many unsatisfactory experiments using dialyzed solutions in which low concentrations of numerous fractions were observed after electrophoretic separation, undialyzed ragweed pollen extracts were studied with more satisfactory results.

Early experiments with dialyzed extracts in the Tiselius cell showed that a highly skin reactive, major, unpigmented, negatively charged, slowly migrating component (USG) was present in giant ragweed pollen extracts. A similar component (USD) was also present in dwarf ragweed pollen. There were usually also six or more minor pigmented components in both types of solutions. The major unpigmented component of giant ragweed extract was present over the pH range of 3.5 to 7.5 without apparent dissocia-

tion. In one experiment, however, the USG fraction showed two peaks at pH 3.5.

The major colorless components are electrophoretically homogeneous. In the ultracentrifuge at 150,000 g they show a single band with the Foucault-Toepler scanning method. As yet, only two fast pigments, FPG and FPD, have been isolated. It is difficult to separate the other minor pigments, because their electric mobilities lie very close together. In physiological salt solution, the colorless components are practically stationary in the electric field. In the presence of less salt, they, as well as the fast pigments, are negatively charged between pH 4.5 and pH 7.5. We have not, as yet, determined the isoelectric point accurately because of the low electric mobilities observed. The USG and USD boundaries moved during ultracentrifugation with almost identical speeds. The sedimentation constants at 20° are about $1.5 \times 10^{-13} cm^{-1}$. This value is smaller than the smallest sedimentation constant in the table of proteins in Svedberg and Pedersen's "Ultracentrifuge." Ultracentrifugation of crude giant and crude dwarf ragweed solutions showed that the main boundary sediments at about the same rate as the USG and USD components. On direct observation during ultracentrifugation, the pigment above and below the boundaries was equal in intensity of color, but did not separate.

The diffusion constants of the USG and FPG components show that the colorless fraction and the fast pigment diffuse rapidly compared with proteins like serum albumin. The curves also provide evidence that homogeneous systems are being observed. The data thus far obtained at 2°C indicate that the diffusion constant for the USG is $1.7 \pm 0.5 \times 10^{-6}$ cm ^{2}/sec. The diffusion constant for the FPG fraction is slightly smaller. The rapidity of diffusion and the slowness of sedimentation indicate that the major colorless component and the fast pigment have molecular weights which are low when compared with ordinary proteins. Calculations based on these values of the constant resulted in a molecular weight of about 5000 for the colorless components.

The homogeneous USG component has been named trifidin. Experimentally, it produces hay fever and asthma. Similar clinical activity was observed for artefolin (the USD component). It is of interest that the fast pigments are also highly skin reactive. Since the fast moving pigment and the slow moving colorless components are both highly skin reactive, it is tempting to speculate that the colorless component has an immunologically active group which is common to and bound to the pigmented components. The nature of the binding would be of the type not broken by the electric field. This hypothesis of a "master" antigen can be tested by suitable immunological procedures. These experiments are in progress with Dr. G. Shwartzman.

The major colorless components are readily precipitated by phosphotungstic acid and the Millon reagent. In the concentrations which we have employed, they show opalescent or delicate turbidity with the usual protein reagents. The Biuret and Molisch tests are positive.

Have we to do with an ordinary protein in the case of these active materials? It is difficult to say. This much, however, is certain. We have isolated substances of comparatively low molecular weight which have certain protein-like characteristics. Whether it is an ordinary protein or whether it is part of a group of substances related to proteins, which are characteristically to be found in the pollen, allergens may only be discovered by further experimentation. Preliminary data obtained with Dr. Moore indicate that during the electrophoretic analysis of timothy grass extract, an electrophoretically homogeneous, highly skin reactive, colorless component may also be obtained. Similarly, in the timothy pollen extracts, there are at least six pigments and the patterns of timothy closely resemble that of the ragweed extract.

All of our findings may now be assembled. The low value of the molecular weight readily accounts for the ease with which the biologically active materials penetrate the skin when the electric field is applied. It also adds clarity to the fact that persons capable of being sensitized to ragweed are so frequently sensitized. This is so because one of the most important factors

in the production of sensitization should be the rapid diffusion of the active allergenic materials, which leak out of the pollen granules and pass through the mucous membranes of the nose and bronchioles into the tissues beneath.

We have not explained, however, why it is that the electric field is so efficacious in forcing the pollen allergens into the skin. You may recall that I have pointed out that the colorless materials move very slowly in the electric field. The pigments move more quickly. But the slow moving trifidin can be introduced in the absence of pigments. To explain this phenomenon, Gorin and I have shown that a hitherto neglected factor (the diffusion forces) may operate in the pores of the skin. Taking these diffusion forces into account simplifies our explanation of the rapidity of movement of the pollen through the pores of the skin, in this case, through the sweat glands. The electric field may electro-osmotically set up liquid bridges in the pores through which appreciable diffusion may be facilitated.

With Dr. Moore and Mrs. Gettner further studies of the physico-chemical nature of other pollen allergens as well as the development of therapeutic technics are planned. On some future occasion I hope to have the pleasure again of addressing this section on the results of this project.

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SECTION OF PSYCHOLOGY

NOVEMBER 17, 1941

DOCTOR CHARLOTTE BUEHLER, College of the City of New York, and Clark University, Worcester, Mass.: *Symbolic Action in Children.* (This lecture was illustrated by charts, drawings and opaque projection.)

The idea of this paper is to connect the demonstration of a new test with the discussion of a fundamental problem in child psychology and psychotherapy: namely, the problem of symbolic action in children.

A symbolic action is an action in which the overt behavior, the direct expression, and the manifested purpose stand for another behavior, expression, and purpose than the manifested ones.

The first question which is discussed is: When does, and when does not, the make-believe play or also any other play mean and express something beyond its admitted, overt, direct content? Erik Homburger Erikson's analysis of the case of Mary[1] is taken as a starting point for the discussion of those features in her play which are so age-characteristic that, without further specific evidence, they cannot be assumed to be an individual expression of an individual problem, as compared with other features in her play which are very individual and which, therefore, are much more likely to express and to mean something.

The second question which is raised is: Which items or features of an action are used for symbols? Content and form can both be used for symbolic expression. Enclosures are used, as an example, for the discussion of a formal symbol and the next question is then raised whether one and the same item expresses always the same or quite different ideas. Ernest Schachtel's study on perception of adults[2] seems to show, correspondingly to play ob-

[1]Erikson, Erik Homburger. Studies in the interpretation of play I. Genetic Psychology Monographs, 140, 22, 557–671.

[2]Schachtel, Ernest. The Dynamic perception and the symbolism of form. Journal of the Biology and Pathology of Interpersonal Relations, 4, 79–96 (1941).

servations with children, that enclosures can mean quite opposite things, namely shelter as well as imprisonment. There are still further meanings in children's use of this symbol.

The last question raised in this general discussion is: Whether every human being has the same disposition to express himself symbolically. Again, in agreement with Schachtel's observations with the Rohrschach Test and with observations of Bender and Schilder[1], the hypothesis is formulated that the threshold for the disposition to produce and to perceive symbols in individually a very different one.

The World Test is briefly introduced.[2] This test originated from Margaret Lowenfeld's World Play, the use of which she described some years ago.[3] The test is based on the analysis of 60 worlds of Viennese school children from 4 to 9 years of age; 25 worlds of Minneapolis kindergarten children of 4 and 5 years; 180 worlds of clinical cases of European and American children from 5 to 16 years.

The test and present analysis makes use so far of 7 characteristics of the worlds. These characteristics are called symptoms because they discriminate significantly problem children and retarded children from normal adjusted children. The seven characteristics so far studied are empty worlds with less than 50 elements; worlds in which only 5 types of elements or less are used; worlds without people; worlds with soldiers or other aggressive elements and with aggressive action like killing, accidents, burying; worlds with certain formal characteristics like rows, enclosures, chaotic distribution of elements and two degrees of organization.

Some of the results which were discussed were: (1) The characteristics called symptoms occur significantly more frequently with

[1]Bender, Loretta, and Schilder, Paul. Form as a principle in the play of children. Jour. of Genetic Psychology 49, 254–261 (1936).

[2]Buehler, Charlotte. Symbolic Action in Children. Jour. of Orthopsychiatry. Manual of the World Test. Edited by Buehler, Charlotte; Kelly, Gayle; and the Psychological Corporation. (In preparation.)

[3]Lowenfeld, Margaret. The World Play. Brit. Jour. of Medical Psychology, 18, 65–101 (1939).

retarded children than with problem children; with problem children than with normal children (ratio roughly 3:2:1); more frequently with younger than with older children; with girls than with boys. This means that they occur in increasing number with increased weakness or incapacity to cope with life. (2) The symptoms seem to be partly unspecific and partly specific. The unspecific symptoms occur with various types of conditions and problems. The specific symptoms occur in significant correlations with certain types of conditions and problems.

Unspecific symptoms are empty worlds which occur also with normal children and which may indicate, in a majority of cases, just lack of imagination and nothing more. Worlds without people and worlds in which fight takes place seem also to occur with almost any type of problem child whose contact with people is disturbed. There are, however, some exceptions to this observation, in that there is a significant negative tetrachoric correlation between hysterical children and worlds without people. That is to say, hysterical children generally do not make worlds without people. There are further positive and negative correlations as regards the number and use of aggressive elements with various types of problem children.

Among the specific symptoms, the enclosures and the chaotic worlds are of outstanding importance. They lead through the study of quite a number of significant correlations to the observation that two main opposite neurotic phenomena can be established. The one is the phenomenon of disintegration and confusion, the other is an opposite extreme, namely, too rigidly keeping together and closing together. One is giving up and losing hold over oneself; the other is protecting and closing off oneself with a certain neurotic strength.[1] These observations in connection with the correlations on which they are based, suggest different techniques in the approach to these two groups of children when they are to be treated.

[1]Saul Rosenzweig considers these observations in good accord with his and David Shakow's findings with schizophrenics. See "Play Technique in Schizophrenia." American Journal of Orthopsychiatry. 7, 31–47 (1937).

SECTION OF ANTHROPOLOGY

NOVEMBER 24, 1941

DOCTOR RALPH LINTON, Columbia University, New York, N. Y.: *Some Recent Developments in Southwestern Archaeology.*

The archaeology of the Southwestern United States has been studied more intensively than that of any other part of North America and a fairly clear picture of the interrelations and developmental sequences of the various cultures present there is beginning to emerge. That early man was contemporary, with a Pleistocene fauna in this region, seems to be proved. However, even the earliest remains indicate the presence of a multiplicity of cultures whose origins and interrelations are still uncertain. These cultures fall into two main groups. One group is characterized by finely chipped stone implements, especially projectile points, among which the Folsom and Yuman types are the best known. Their economy seems to have been based upon big game hunting. The other group shows a striking lack of finely chipped implements, especially in the lower levels, but is characterized by the presence of great numbers of grinding stones. Their economy seems to have been based upon small game hunting and seed gathering. The distribution of these two groups in the Southwest is still imperfectly established but the seed gathering cultures seem to have been most strongly developed in the southern and western parts of the area. The big game hunting complex, on the other hand, seems to have been most strongly developed on the northeastern borders of the area and is linked with the earliest cultures of the high plains. The presence of occasional implements of big game hunting types in sites of the seed gatherers indicates that the two complexes were contemporaneous, while the same Pleistocene fauna is associated with both.

No connections have so far been established between the cultures of the big game hunters and the later agricultural peoples of the region. One of the seed gathering cultures, the Cochise,

shows many resemblances to a later agricultural culture, the Mogollon. Since the latter dates from only slightly before the beginning of the Christian era, late survival of the earlier culture is suggested. The similarity of implement types throughout all Cochise horizons is so marked that it seems improbable that this culture has the great age assigned to it by geologists.

The later agricultural cultures of the Southwest can now be reduced to three main groups with a possible fourth. The established groupings are the Hohokam, Mogollon, and Anasazi. The Hohokam centers in southeastern Arizona. Its origins are obscure, but it shows, from the earliest levels, numerous resemblances to the higher cultures to the south, in Mexico. The Mogollon also centers in Arizona, but to the north and east of the Hohokam area. It seems to be the earliest agricultural, pottery-using culture in the Southwest and to have developed out of the local Cochise culture. Mogollon sites are strictly linked with an environment suited to the growing of maize without irrigation or the development of drought-resisting varieties, i.e. well adapted to the beginnings of its cultivation, when it was first diffused from Mexico. In spite of its early beginnings and influence on neighboring cultures, the Mogollon culture never developed great complexity and was eventually absorbed by the expansion of Hohokam and Anasazi. The Anasazi group centers to the north and east of the Mogollon and has been studied more intensively than any of the other groups. It begins with the maize-cultivating but potteryless people of Basket Maker II and culminates in the great communal dwellings of the Chaco and Mesa Verde. There are many indications that it received the idea of pottery-making from the Mogollon.

The possible fourth group of cultures centers on the northeastern borders of the Southwestern area. First recognized in one of its later phases, the Gallina, Columbia University's work of the past summer, has pushed this line back to about 700 A. D. Even then, it possessed full agriculture and pottery. Sites of this culture in the Gobernador region of northern New Mexico are characterized by small settlements consisting of one to at most four or five

dwelling complexes. Each complex was presumably occupied by some sort of kin group. It has as its central feature a very large and deep circular pit house with small central fireplace, banquette and ventilator shaft, reminiscent of the kivas of the later Anasazi cultures of this region. Associated with this, on the surface, were rows of small rooms, apparently used as granaries, numerous fireplaces and shades. Some pit houses have associated with them small, shallowly excavated circular structures suggestive of menstrual lodges. The whole site is commonly surrounded by a fence or stockade. All the pit houses excavated had been burned after the interiors had been put in order; metates placed in one part of the house; pottery in another, etc., suggesting some sort of ritual destruction. All burials found were secondary, after the exposure of the body for some time. Most of the small bones were missing, while one skull contained a wasp's nest.

Pottery is of two types: bowls of a painted ware closely related to the contemporary Basket Maker wares and cooking pots and jars of a coarse dark grey ware apparently not related to Basket Maker. The latter ware was always made in baskets about two inches high, the walls of the vessel being carried up above this by coiling. The exterior was frequently rubbed with a bunch of grass or pine needles giving a striated surface. Agriculture is proved by finds of charred maize.

This early phase seems to pass through gradual modifications in house types into the typical Gallina. In this level, the painted ware still shows strong similarities to the contemporary Anasazi wares, but the utility ware ceases to be made in baskets and assumes a form very similar to that of the historic Navajo utility wares.

The painted wares of this group of cultures are clearly dependent upon Anasazi influence throughout their history. The utility pottery, on the other hand, shows strong resemblances to coarse wares, whose distribution extends northward and northeastward into the Plains and Plateau. It is characteristic of these coarse wares that they were employed almost exclusively as cooking pots. This use of pottery exclusively as a food-boiling ap-

pliance, bowls, spoons, etc. being made of other materials, is characteristic of the far northern pottery using cultures of both the Old and New Worlds. The rarity and small size of cooking vessels, as distinct from bottles, bowls, etc., in the Basket Maker III culture, suggests that a stimulus toward food-boiling may have come into the Anasazi line from some northern culture, bringing with it a distinctive type of utility pottery. The sharp distinction in surface and paste, which distinguishes Anasazi cooking wares from other wares in simultaneous use, may reflect an original diversity of origin.

It is suggested that the appearance of the Gallina line in the Southwest may be correlated with the intrusion into the area of some northern group, who brought with them, not only a new type of utility ware, but also the deep pit house, a widely distributed circumpolar feature. The sudden appearance of this structure, which has no Basket Maker prototype, and its persistence in later Anasazi cultures as the kiva, may be a result of borrowing.

It is tempting to assume that the people of the Gallina line were the ancestors of the later Navajo, but the only close similarities between the two cultures are those found in the utility pottery. The gap between the latest Gallina and the earliest Navajo sites still remains to be bridged.

SECTION OF PHYSICS AND CHEMISTRY

NOVEMBER 14 AND 15, 1941

Conference on "*The Ultracentrifuge.*"

The Section of Physics and Chemistry held a Conference on "The Ultracentrifuge", the first of the series this year. Doctor Duncan A. MacInnes, The Rockefeller Institute for Medical Research, New York, N. Y., was in charge of this meeting, as Conference Chairman.

The program consisted of the following papers:

"Introductory Remarks," by Duncan A. MacInnes.

"The Production and Maintenance of High Centrifugal Fields," by J. W. Beams, Rouss Physical Laboratory, University of Virginia.

"Optical Problems of the Ultracentrifuge," by J. W. Williams, University of Wisconsin.

"The Integration of the Differential Equation of the Ultracentrifuge," by W. J. Archibald, National Research Council, Canada.

"The Present Status of Molecular Weights of Proteins," by Alexandre Rothen, The Rockfeller Institute for Medical Research.

"The Effect of Centrifugal Fields on the Electromotive Force of Galvanic Cells," by Duncan A. MacInnes.

NEW MEMBERS

Elected November 3, 1941

LIFE MEMBER

Evans, Florence L., Ph.D., Associate Bacteriologist, U. S. Marine Hospital, Carville, La.

ACTIVE MEMBERS

Collins, Robert E. Lee, Ph.D., Associate Professor, Geology and Geography, University of Tennessee, Knoxville, Tenn.

Conroy, Peter J., Ph.D., Professor, Chemistry, College of Pharmacy, Fordham University, New York, N. Y.

Coons, Robert Roy, Ph.D., Head, Natural Sciences, George Pepperdine College, Los Angeles, Calif.

Cooper, Charles L., Ph.D., Professor, Industrial Education, Agricultural and Technical College, Greensboro, N. C.

Cooper, Stewart Rochester, Ph.D., Assistant Professor, Chemistry, Howard University, Washington, D. C.

Corlis, Edwin Fleming, Ph.D., Professor, Biology, St. Francis College, Brooklyn, N. Y.

Craig, Gladys M., M.Sc., Bacteriologist and Protozoologist, Doctor's Hospital, New York, N. Y.

Crouse, Helen V., Zoology, Columbia University, New York, N. Y.

Dante, John Henry, M.S., Paleoichthyology, Zoology, Washington, D. C.

De Leon, Benjamin, M.A., Teacher, Physics, South Side High School, Newark, N. J.

Eddy, Bernice Elaine, Ph.D., Associate Bacteriologist, Division of Biologics, National Institute of Health, Bethesda, Md.

Ellickson, Raymond T., Ph.D., Instructor, Physics, Polytechnic Institute of Brooklyn, Brooklyn, N. Y.

Ellis, Emory L., Ph.D., Instructor, Biochemistry, California Institute of Technology, Pasadena, Calif.

Fay, Temple, M.D., Professor, Head, Neurology and Neurosurgery, Temple University, School of Medicine, Philadelphia, Pa.

Felton, Lloyd D., M.D., Sc.D., Senior Surgeon, National Institute of Health, U. S. Public Health Service, Washington, D. C.

Fosbinder, Russel J., Ph.D., Director of Research, Vice-President, The Maltbie Chemical Company, Newark, N. J.

Foxe, Arthur N., M.D., Instructor, Neurology and Psychology, Bellevue Medical College, New York, N. Y.

Francis, Thomas, Jr., M.D., Sc.D., Professor, Chairman, Epidemiology, University of Michigan, School of Public Health, East Hospital, Ann Arbor, Mich.

Gardner, William U., Ph.D., Associate Professor, Anatomy, Yale University, School of Medicine, New Haven, Conn.

Gollomb, Joseph, M.A., Writer, Psychology, New York, N. Y.

Goodfellow, Thomas John, M.D., Surgeon, Biology; Eye, Ear, Nose and Throat Division, Saratoga Hospital, Saratoga Springs, N. Y.

Goorley, John T., Ph.D., Director of Research, Pharmacy and Medicine, Lex Laboratories, Habana, Cuba.

Gordon, William Henry, M.D., Chief of Staff, Internal Medicine, North End Clinic, Detroit Mich.

Gray, Edward LeB., Ph.D., Research Biochemist, Lever Brothers, Wellesley Hills, Mass.

Gruber, Rudolf E., Sc.D., Chemistry, Biology, Vice-President, Merck and Company, Inc., Rahway, N. J.

Gruse, W. A., Ph.D., Industrial Research, Chemistry, Petroleum Technology, Mellon Institute, Pittsburgh, Pa.

Shoemaker, Norman Clifford, M.D., Staff Member, Biology and Chemistry, Doctor's Hospital, New York, N. Y.

Storandt, Kenneth M., M.S., Psychology and Anthropology, Faculty, New York School of Social Work, Columbia University, New York, N. Y.

ASSOCIATE MEMBERS

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Brussel, James Arnold, M.D., Chief Neuropsychiatrist, Station Hospital, Fort Dix, N. J.

Cooper, Frank L., Ph.D., Instructor, Sloane Physics Laboratory, Yale University, New Haven, Conn.

Cruess, William V., Ph.D., Professor, Food Technology, University of California, Berkeley, Calif.

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Hall, David G., B.S., Associate Entomologist, U. S. Department of Agriculture, Bureau of Entomology and Plant Quarantine, Washington, D. C.

Hanmer, Hiram Rupert, B.S., Director of Research, Chemistry, American Tobacco Company, Richmond, Va.

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